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Phylogeography and evolutionary aspects of Indian fishes: Challenges for the future*

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ABSTRACT

A primary goal of historical ecology is to determine how communities and biological interactions develop. Interactions among species may vary geographically based on the ecological context and such variation may arise in very recent species associations. Comparative phylogeography represents a solid approach to study both the historical and ecological contexts of these interactions. Phylogeography is a field of study concerned with the principles and processes governing the geographical distributions of genealogical lineages at the inter- and intra-specific level. Mitochondrial DNA (mtDNA) has been used extensively in the last three decades as a tool for inferring the evolutionary and demographic past of both populations and species. This tool has proven invaluable for the new fields of molecular ecology and phylogeography. With the development of DNA sequencing methods and the extensive sequencing experiments undertaken in the last two decades in a wide variety of organisms, a great number of phylogenetic studies using mitochondrial gene sequences have been carried out and reported, some dealing with the use of mitochondrial genes in the establishment of different levels of phylogenetic relationships. Many studies using mtDNA as an evolutionary marker are now considered modern literature classics and demonstrate that deep insight can be obtained from studying mtDNA. Starting late in the 19th century till the first half of the 20th century, biogeographers in India were attempting to explain the apparent discontinuity in the ranges of certain plants and animals. The isolated presence of flora and fauna of Malayan origin in the Western Ghats and other south Indian hills led to a lot of speculation on the pre-history of peninsular India. Many hypotheses have been proposed and Dr. Sunder Lal Hora's, "Satpura Hypothesis", found wide acceptance. In the light of the recent advances in molecular systematics and ecology of freshwater fishes, the paper reviews the current status of information on the genealogical lineages of Indian fishes, goal and tasks of genetic management of natural fish populations; and closes with identifying future challenges in the field.

Key words: Evolution, Fishes, India, Phylogeography

We must be thankful to Carl Linnaeus who in his magnum opus *Systema Naturae* (1758) gave us the binominal nomenclature defining the bio-unit we call as species in animals and plants. The last two and a half centuries have seen a lot of refinements in taxonomy, where with the traditional system, we blend new concepts in the genetic make up of the species to give us a clear vision of how best species and intra-specific categories can be delineated. The Galapagos Islands surveyed and researched by Charles Darwin threw up many explosive ideas on the origin of species, natural selection, adaptive radiation and so on. Today, about 90,000 pages of manuscripts, field notes, and sketches connected with Charles Darwin are online for free viewing. Ichthyologists and scientists in general do not tend to stray outside the sphere of their special subject interest. I belong to an old school where reading for pleasure beyond the subject area of work is an ingrained habit. Since I mentioned Galapagos Islands, I would like all to read the classic work of David Lack on "Darwin's Finches" which exemplifies how in an insular area isolated from the mainland, the finches have adapted themselves to different niches in the island ecosystems resulting in speciation *par excellence*. The blending of species with the different ecological situations shows how nature works without human interference.

The historical perspective of the ichthyofauna of India shows a chequered bio-geographical past. Global palaeomaps show the disposition of India in relation to other Gondwana continents and Eurasia in the Mesozoic. Briggs (2003a) in a review of the biogeographic and tectonic history of India, basing his findings on stratigraphy, palaeomagnetics and palaeontology speaks of how India-Madagascar rifted from East Africa 158–160 ma (million years ago); India-Madagascar from Antarctica about 130 ma; India- Seychelles from Madagascar 65 ma; India colliding with Eurasia about

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65ma and final structuring taking place 42 – 55 ma ending the Tethys Strait across Northern India. India's proximity to Africa and Madagascar during the northward movement facilitated faunal exchange. Briggs (2003b) terms the drifting Indian landmass as a "biotic ferry" and an "evolutionary reservoir" for Gondwana groups. This suffices to explain the genealogical patterns of groups with recent distribution in Africa, Madagascar and India. Such a concept also suggests that lineages also colonized South and South-East Asia "out of India" (Karanth, 2006; McKenna, 1973). A significant group of fishes which needs detailed study for speciation are the Cichlids of India and Madagascar, while their counterparts have diversified to several hundred species in the great lakes of Africa. The species from India and Madagascar got separated from the African species about 165m years ago followed by a split between India and Madagascar about 90ma. It will be necessary to have an in depth look at the molecular phylogeny of the Indian endemic Cichlids, now known from only three species, *Etroplus suratensis, E. maculatus and E. canarensis.* A similar, but hitherto un-noticed group of fishes are the blind clariids of Kerala, India (*Horaglanis krishnai* and *H alikunhii*) and Africa. These blind catfishes live in the subterranean aquifers and we have touched only the tip of the iceberg. A systematic study of this group is called for as I feel many more genera and species may await discovery. Molecular phylogenic studies of blind clariids should throw open new vistas on the evolutionary history of Indian fishes.

In "*Population, Species and Evolution*", Ernest Mayer (1963) opined that in allopatric model of population dynamics, genetic drift accounts for a gradual divergence of population combined with selection during extended periods of separation or isolation by physical barriers to gene flow. Palaeogeolological events in the past have played major roles in the distribution, abundance and pockets of great species diversity in South – East Asia. However, the role of physical barriers, (some of which may not exist today, or, may have become enhanced manifold times), in speciation are not well understood. Eustatic movements of sea level would be one such barrier for freshwater fishes during the pluvial periods of the Pliocene- Pleistocene.

Satpura Hypothesis: Our knowledge of the biogeography of India, especially the Ichthyogeography was enhanced by the work, zeal and efforts of Dr. Sunder Lal Hora (Fig. 1) who propounded the Satpura Hypothesis to explain the biodiversity and Malayan affinities of the faunal and floral elements along the watersheds of the Western Ghats of South India, North East India, [two of the 34 biodiversity hot spots in the world (Viswanath *et al. 2007*; Ponniah & Gopalakrishnan, 2000)] and the Malay Peninsula. In present day parlance, it represents "into India" migration from the focal area of Yunnan in China passing through N.E. India, the Garro gap and along the Narmada-Tapti watersheds draining the Satpura mountain ranges and on to the Western Ghats. Another lineage passed through the Irrawaddy System of Myanmar southwards to Malaysia, geological events and changes in watershed drainage patterns aided such migrations. In my work on "Classification, zoogeography and Evolution of cyprinoid families Homalopteridae and Gastromyzonidae" (Silas, 1953a) I had occasion to study in detail the Malayan affinities of these fishes in relation to those of the Western Ghats. A very interesting aspect was the connectivity of the Sundaland based on glacio-eustasisms when river systems of Malaysia and Indonesian islands, west of the island of Bali, were linked. The Wallace line between Bali and Lumbok islands separated the faunal and floral elements of the Sundaland from that of Papua New Guinea and Australia. Eustatic moments during the interglacial periods resulted in the present day disposition of these land and river basins. A similar connection between Sri Lanka and South India could have existed at that time accounting for the great similarities in the fish faunal elements of the Western Ghats and Sri Lanka. With respect to freshwater teleost species, the streams and rivers originating from the Western Ghats and Sri Lanka are two of the few sites in the world exhibiting high degree of endemism and exceptional biodiversity. More endemic fishes from the Western Ghats have been recently reported by Ponniah and Gopalakrishnan (2000).

I recollect the late forties and early fifties as an exciting period when Hora could elicit multi-disciplinary interaction from geologists, palaeontologists, palaeobotanists, palaeogeographers, besides botanists, mammalologists, ornithologists, herpetologists, ichthyologists and scientists from other disciplines to get together and discuss issues relating to his Hypothesis. Sad to say, the last fifty odd years have not witnessed any such inter-disciplinary concerted action on biogeographical problems. It was a rare privilege for four of us, A.G.K. Menon, K.C. Jayaraman, T.V.R. Pillai and myself to be associated with Hora at this crucial time of the explosive growth of taxonomic and biogeographic interest. My contributions were on the taxonomic assessment and evolutionary divergences of the fishes with the so-called Malayan affinities in Peninsular India (Silas, 1952) and also on insular speciation among the freshwater fishes of Ceylon (presently Sri Lanka) (Silas, 1953b). At that time we lacked genetic tools to evaluate species diversity and intra-specific levels of diversity. Two excellent and pioneering works by Pillai (1951) on racial studies in *Hilsa ilisha* and *Puntius sarana* were mainly based on morphological and meristic characters. Probably the time is now appropriate to explicitly reconsider some of the issues discussed on the influx of species and speciation in time and space using genetic markers. It is in this context that *genetics* has an important role to play in the study of Indian biogeography.

Natural fish populations are declining at an alarming rate in many parts of the world due to over-fishing and other man made activities. The very sustainability of fisheries resources

are being affected and their gene pools and genetic diversity being eroded. Rapid advances in molecular biology have helped to develop "molecular markers" in the form of Restriction Fragment Length Polymorphism (RLFP) of nuclear and mitochondrial DNA. In a milestone publication entitled "Intraspecific phylogeography, the mitochondrial DNA Bridge between population genetics and systematics", J.C. Avise and Colleagues (1987) proposed the term "Phylogeography" as a discipline involving biogeography and population genetics. Phylogeography, by definition is the use of tools of molecular biology, especially the mitochondrial DNA (mtDNA) to infer phylogenetic histories of taxa. It could help discern the evolutionary processes that generate biodiversity. More recently, Bermingham and Moritz (1998) opined that comparative phylogeographic analyses could permit detailed studies of "landscape evolution, including the dispersal of fauna through a region, speciation, adaptive radiation and extinction" besides help molecular genetics for fisheries management and conservation, especially of threatened species. Phylogeographic reconstruction could show how species have originated by range expansion in time and space, and has facilitated genealogical traces to be followed across genetic boundaries between populations, species and higher taxonomic levels. With the advent of polymerase chain reaction (PCR), some quantitative changes in the approach of studying inter-population genetic variation gained momentum. The availability of nucleotide sequence data has helped to develop universal oligonucleotide primers to amplify specific regions of mitochondrial DNA. The Randomly Amplified Polymorphic DNA (RAPD) technique using random oligonucleotide primers (Williams *et al.* 1990) became useful for stock identification studies. DNA fingerprinting by using minisatellites and especially microsatellite markers provided finer resolution. Since these repetitive DNA regions are not under the stringent control of natural selection, they generally show higher level of genetic divergence at the nucleotide sequence level. These markers are useful in detecting the population and identifying individuals (Zardoya *et al.* 1996).

With the facilities, infrastructure and trained manpower available with us today, I would call for a more intensive study of the biodiversity of our fishes in the wild as well as commercially important species, whether in aquaculture or in capture fisheries, adopting a phylogeographic approach. This could conceptually bring about a new look of our species in the light of barcoding, aquaculture, green certification, trade and related aspects where precise nomenclature becomes essential. This cannot be done at a leisurely phase as most of our freshwater, brackishwater and coastal aquatic ecosystems are under ever increasing pressure from anthropogenic activities. I would like to cite one good example of results of concerted action. As recent as 2002, over 100 species of Racophorine tree frogs were described in Sri Lanka using mtDNA in combination with exophenotypic measures, when only 18 species were previously known. Many more could have become extinct through human activities (Megaskumbura *et al*. 2002). The last said is true for the Indian scenario as well. Let us not forget that any work on Phylogeography should have a multidisciplinary approach and knowledge of biogeography, ecology, behaviour and other aspects of the species or population.

In the ocean environment though there appears few physical barriers and larval dispersal in general are extensive, yet, the tropical seas and ecosystems such as the coral reefs evince high species diversity which has puzzled those involved with allotropic species models. Studying the wrasses genus *Halichores,* Rocha *et al*. (2005) found strong partition between adjacent ecologically distinct habitats (in *H. vittatus* and the species pair *H. radiatus*/*H. brasiliensis* they observed 3.4% and 2.3% divergence respectively) "but high genetic connectivity between similar habitats separated by thousands of kilometers." According to them, "The concordance of evolutionary partitions with habitat types, rather than conventional biogeographical barriers, indicates parapatric ecological speciation, in which adaptation to alternative environmental conditions in adjacent locations overwhelm the homogenizing effects of dispersal." This probably solves the puzzle about the high biodiversity of coral reef fauna. Other recent studies also show the local retention of reef fish larvae (Jones *et al*. 1999; Swearer *et al*. 2002), active habitat choice by larvae (Bierne *et al*. 2003) and reduced gene flow over short geographical distances (Taylor & Heilberg, 2003) indicating the possibilities that ecological partitions can drive speciation, especially when contrasting environments are in geographically separated, but potentially connected locations (parapatry) leading to high biodiversity in coral reefs.

Determining the status of a taxon: The status of a taxon can be understood by surveying its range of distribution, abundance and population composition; and decision can be taken accordingly for its conservation management. The basic knowledge of its taxonomy, biogeography, life history characters such as age structure, fecundity, spawning behavior and running time in the case of migratory species and straddling stocks will be of immense help. All these information can be obtained from scientists, naturalists, conservationists and the folk who are the traditional users of the resource, and documented. The technical information available on the structure of the population should be catalogued preferably by computerized data base for quick retrieval and exchange of information among the scientists and the managers. The status of a taxon can be ascertained based on the above data base and conservation programmes designed accordingly.

Importance of stock identification: Stock identification will improve our understanding about the genetic structure

of natural populations. The determination of genetic variation within and between populations can discriminate between genetically poor and rich population in terms of heterozygosity and polymorphism. This information will help to decide the best source of material for introduction or rehabilitating a threatened stock.

The stocks are believed to be locally adapted populations. Therefore, they should be treated as the unit of conservation and the management of endangered and commercially important taxa. Phylogeography could help a stock to be labeled to its geographical origin. This could help in understanding the migration pattern between populations and deducing past events of colonization.

Hybrid identification and introgression detection: Significance of hybridization and introgression as a source of gene flow between taxa, especially at the intra-specific levels involving sympatric subspecies and sibling species need our attention. Finding out species specific molecular marker is essential for identification of F_1 and later generation hybrids. Morphological (morphometric and meristic) characters based on the assumption of phenotypic intermediacy in the hybrid to that of the paternal species have been traditionally used for F_1 hybrid identification. These morphological characters are most often not reliable for correct identification. Moreover, the later generation hybrids cannot be detected by this method.

Chromosome number and structure analysis is an approach. Chromosome studies have been used in the analysis of hybrid members of *Salmonidae, Esocidae, Cyprinidae,* and *Cyprinodontidae*. However, to identify the hybrid between the species possessing identical chromosome number as in the case of Catla, Rohu and Mrigal with 2n = 50, studying the chromosome morphology (the number of metacentric, submetacentric and telocentric chromosomes that constitute the karyotype in a species) would be essential. Since chromosomes in fishes are small in size and classifying them by centromeric position is a subjective exercise, it would be difficult to measure the chromosome arms accurately. The chromosome information, therefore, is of limited use in determining the hybrids. The fluorescence in situ hybridization technique (FISH) is potentially a powerful technique that may find greater application in future to characterize the species and distinguish the F_1 and F_2 hybrids (Phillips and Reed, 1996).

Electrophoretic analysis of informative allozyme loci with fixed allelic differences between species can be analysed to identify hybrids. By using six or more species specific unlinked isozyme gene loci, it is possible to discriminate F_1 and post-F_1 fish hybrids accurately. Nuclear DNA RFLP shows biparental inheritance in a Mendelian fashion. This method can be used for both species and hybrid identification. Mitochondrial DNA RFLP/sequence data of selected genes such as 16S rRNA is useful in identifying the maternity of the hybrid in conjugation with nuclear DNA or isozyme markers. Mitochondrial DNA can also be used for studying the direction of hybridization in natural populations and detecting the occurrence of introgression. Inadvertent hybridization of Indian major carps has been detected in hatcheries using mitochondrial DNA RFLP (Padhi and Mandal, 2000). Occurrence of hybrids in nature is not uncommon. I have the experience of dealing with an enigmatic specimen of tuna from off Mangalore which apparently could be a hybrid between *Euthynnus affinis* and *Katsuwonus pelamis* but still doubt persists (Silas *et al*. 1981). In such cases molecular techniques may be highly useful.

Determining the genetic problems: To determine the genetic problems, *gene pool monitoring* is essential. Having prior knowledge about the status of a taxon and its genetic diversity, the geneticist can determine the type of genetic problems to resolve through development of sound management strategies. The following questions may be addressed to find out the genetic problems:

- Has population size reduced? If yes, the occurrence of inbreeding and genetic drift is probable, which can be ascertained by heterozygosity and polymorphism analysis.
- Is inbreeding between different stocks going on due to purposeful introduction and inadvertent escapement? If yes, does genetic admixture lead to genetic contamination?
- Does inter-specific hybridization occur between closely related species? If yes, is genetic pollution occurring due to genetic introgression?
- Does chemical pollution (at a lower dose) affect fish gene pool by causing genetic toxicity?

Conservation approaches: Once the goal and tasks of conservation are decided, a specific management approach can be designed. The genetic goal of a conservation programme is to conserve the genetic diversity, though inherently it is very complex. Hence, considering it as components would be useful in conservation planning. For this it is essential to address three fundamental questions: What to conserve?, Where to conserve? and How to conserve? Let us look at this more critically:

What to Conserve?: Protecting an ecosystem may be a method of conserving "everything". This approach is broad based, non-specific, cost effective and relatively simplistic. No special knowledge is required of the biology and genetic diversity of a species for conservation management. This may be advantageous in view of our inadequate knowledge of the genetic diversity and its potential or actual value. However, since the role of a particular species is ignored in favour of ecological process and community organization, this approach may prove ineffective, for the conservation of an endangered species. Conservation may aim at a "specific species". A species becomes prominent in conservation planning for a number of reasons: i) when it is declining due to anthropogenic stress in natural waters, ii) when it is crucial

for the well being of its ecosystem, or iii) when it is endangered and chosen for recovery by special management measures. To conserve a declining species, we should have sound knowledge about its biology, biogeography, and genetic diversity. Without proper knowledge, inter-population genetic diversity cannot be conserved.

Where to conserve?: Conservation can be done *in situ* in a safe refuge or *ex situ* in the laboratory. *In situ* conservation means conserving the whole ecosystem or the total community in its natural location without any specific attention on any particular species. However, when a species is of special concern *in situ* conservation could prove inadequate. Habitat degradation could affect the reproduction of a species when *ex situ* conservation becomes an option. Developments in biotechnology have made it possible for cryopreservation of spermatozoa in sperm bank and work on embryo preservation is also being attempted. The germplasm can also be stored in the laboratory in the form of DNA Bank as i) total genomic DNA, ii) in the form of DNA library *ie*., genomic DNA or cDNA library or iii) as cloned DNA fragments. This would also require proper documentation, labeling and proper preservation and technical expertise to handle the same. Since the stored DNA may be useful for the recovery of some genes and not the genome as a whole, it may be helpful for research use, and cannot replace the natural genetic diversity.

How to conserve?: This may have two aspects, one managing declining population and the second, managing endangered species. For *managing the declining population* some corrective measures based on the following genetic principles may be an answer. i) the effective population size should be maintained as large as possible to maximize the contribution of a large number of adults for reproduction, ii) the causative factors that reduce the effective population size should be controlled. If there is a genetic bottleneck, the duration should be reduced as far as practicable, and iii) the barriers that create discontinuity in an inbreeding population should be disrupted to maintain continuity of gene flow. For this it will be necessary to protect the species and habitat *in situ* from anthropogenic stress, by actions such as, imposing ban on fishing during breeding season, gear and mesh size regulation, and regulated well monitored fishing for maintaining the population size. In rivers, stretches may be declared as sanctuaries. To make this work, stakeholder and public participation will be essential. If the population density is critically reduced, supportive breeding for conservation management maybe necessary. But this would need a cautious approach as only a small fraction of the population is allowed to produce progeny for the next generation.

In the second, namely *managing the endangered species,* the population size being small, inbreeding and genetic drift are common genetic problems. Captive breeding is an useful approach for the conservation of endangered species facilitating rapid growth of the population to enhance genetic variability. Use of cryopreserved spermatozoa would be an useful way for increasing the effective population size and recovery of a severely endangered population. Tomoyuki *et al.* (2006) developed the first germ cell transplantation in lower vertebrates using fish PGCs and spermatogonia. "In fish germ cell transplantation system, donor cells are microinjected into the peritoneal cavities of newly hatched embryos" allowing "the donor germ cells into migrate towards, and subsequently colonize, the recipient genital ridges. The recipient embryos have the immature immune systems so the donor germ cells can survive and even differentiate into mature gamete their allogenic gonads, ultimately leading to the production of normal offspring" (underlining mine). "The results of the transplantation studies in fish are improving our understanding the development of germ cell systems during vertebrate evolution". This indicates new vistas open for multidisciplinary approaches in the filed of germ cells transplantation techniques, which the Bureau may consider in its Vision.

Threats to fish genetic diversity: Habitat alterations due to deleterious effects of pollution, damming of main rivers, siltation, introduction of non-indigenous species (exotics), networking and linking of river systems and waterways, all have serious impact on native wild fish populations. The extent and magnitude of such impacts needs to be genetically addressed. Phylogeography could be the tool towards conservation of endangered and threatened species.

Introduction of exotic species: **"**Exotic" is the term used to indicate species living outside its natural geographical range. Terms such as "introduced", "non-native", "non-indigenous" "alien" or "invasive" are also used to denote exotics. In aquaculture such introductions are for improving productivity or control undesirable aquatic organisms or for recreational purposes. In many cases such instances have proven disastrous. In India, the introduction of the common carp into Kashmir Valley has affected the indigenous *Schizothoracinae* fishes. In Gobindsagar Lake, the indigenous *Catla catla* was replaced by the exotic silver carp (Padhi and Mandal, 2000). Exotic ornamental fish such as the green sword tail (*Xiphophorus helleri*), armoured catfish and African catfish *Clarias gariepinus* have been reported from natural waters of Kerala. Sreenivasan (1995) reported introduction of non-native Chinese and Indian Major Carps as the major factor leading to the decline of endemic Peninsular carps such as *Cirrhinus cirrhosa*, *Labeo kontius*, *Puntius carnaticus, Puntius dubius* and *Puntius pulchellus* in South Indian Reservoirs.

Stock introduction: To augment fish production in reservoirs and rivers, non-native/cultured stocks are often transplanted. The cultured stock which genetically differs from its wild relatives, may sometimes escape from ponds or cages into natural waters, creating an opportunity for inter-breeding between non-native/cultured stock and native/wild stock. We have not hitherto conducted impact studies of

hatchery stocks or wild relatives, especially the impact of hatchery reared Indian Major Carps. The genetic admixture due to the un-thoughtful stock transfer from one region to the other may also be detectable. Ignorance of the genetic population structure may result in loss of genetic diversity, reducing productivity and damage to the ecology. Knowledge of the size of the component population becomes essential when we have to go back to the wild stock for replenishing brood-stock for aquaculture.

Genetic impact of Introduction: The impact of introduction of exotics is a matter of concern because of ecological and genetic reasons. Predation on native species or competition and spreading of pathogens or parasites are some common ecological concerns. In short, genetic impacts could result in reduction of 'effective population size' by the ecological and other effects of introduction and also alter or make extinct the gene pools of the species/stocks by cross breeding/ hybridization and backcrossing. In some cases, stock transfer which initially appeared to be beneficial turned out to be bad in the long run. We have a lot to research to do in this area.

Future challenges

- To study the phylogeography of the commercially important teleosts and shellfishes with a view to understand distinct stock-structure of the following species for appropriate management decisions.
  - Bombay duck (*Harpadon nehereus*) and *Hilsa ilisha* from different populations.
  - *Etroplus suratensis*, *E. canarensis* & *E. maculatus* – (the only Gondwanan teleost forms in the whole India) – from Kerala, Sri Lanka & the introduced populations form East coasts of India
  - Endemic species of the Western Ghats and NE: 1) *Silurus wynaadensis* (Kerala) & *S. morehensis* (Manipur); 2) *Neolissochilus wynaadensis* (Kerala) and *N. hexagonolepis* & *N. spinulosus* (N.E.) 3) *Tetraodon travancoricus, Carinotetraodon imitator* & *Tetraodon cutcutia.*
  - Detailed Phylogeography of all the Mahseers of India to be worked out.
  - The lonely schizothoracid fish of the Western Ghats – *Lepidopygopsis typus* with other snow trouts of Himalaya.
  - Phylogeographic studies & species diversity of freshwater crustaceans – so far no reports from India other than on *M. rosenbergii*. Another potential species, *Macrobrachium lar* is found only in Andaman & Nicobar Islands - Comparing this stock from that from East of Wallace Line.
  - The only true tuna in our coastal waters, *Thunnus tonggol* which has very disjunct distribution, along the West Coast and Gulf of Mannar and in Australia.

Fragmentation and fusion of palaeo-drainage systems may be an important factor generating current patterns of genetic and species diversity in hill-stream associated organisms. We may have to combine traditional, molecular-phylogenetic, multiple regression, nested clade and molecular demographic analyses to investigate the relationship between phylogeographic variations and hydrological history of drainages in South India and North-East India.

DNA barcoding: Sequence information of selected mitochondrial genes such as 16SrRNA, *Cyt b* and COI (chloroplast genes in plants) has been found extremely useful in resolving taxonomic ambiguities and in describing new eukaryotic species. The last four years have seen a very significant development of "DNA barcoding" using COI sequence data for identifying species. This has great urgency as many habitats are under great stress from anthropogenic activities and there are estimates of the loss of several thousands of species of organisms every year. This should complement conventional taxonomy and help us document our aquatic (marine and freshwater) biodiversity and help in conservation management. In the production systems we are today looking at quality products in a value chain mode. Hence, traceability of fish and fish production becomes vital. DNA Barcoding can play a major role in preventing the adulteration of fishery products. I am glad that Dr. Lakra has taken the lead in this mission oriented task and wish him and his colleagues all success. However, as mtDNA is only maternally inherited, to avoid ambiguity, sequence information of an ideal single copy nuclear gene such as RAG2 or Rhodopsin may also be used in addition to mtDNA for species level identification.

Emphasis on marine sector: Phylogeography and phylogenetics of our coral reef, mangrove and sea grass ecosystems and the fishes and invertebrates associated with them are under pressure from manmade activities and natural phenomena and climate change need special attention for documenting them. Genetic analysis using appropriate markers such as microsatellites could elucidate the genetic variations in species at the intra-populations levels. Improper management and misjudged priorities in the marine sector has resulted in the decline of major fisheries resources which are replaced today by less value fish. We have hardly any genetic information on species and at intra-specific levels to know the extent to which populations and population segments have been wiped out or have reduced genetic diversity within populations. Loss of genetic viability associated with over-fishing of specific species in commercial operations need investigation. Greater attention should be paid on behavioral ecology, speciation and analysis of social structure of freshwater fish species.

"Phylogeography" of NBFGR: I wish to express my great pleasure that during the last 25 years NBFGR has developed as an unique Institution devoted to research on fish genetic resources, probably the only one of its kind. This speaks a lot about the Directors and staff who have helped to mould it

as a Centre of Excellence in fish genetic research and I have satisfaction in seeing the Bureau develop from strength during.

My memory goes back to the early 1980's when I had prepared a plan for the establishment of a Bureau of Fish Genetic Resources at the Central Marine Fisheries Research Institute, Cochin, Kerala and the Government had sanctioned Rs.22.67 Lakhs for setting up the Bureau in the 6th Five Year Plan period as a Project with a Centre at CMFRI. The Bureau was visualized as an agency to collect and collate information regarding the genetic resources, particularly of culture and commercial value. In perspective, the Bureau was to be the nucleus of a full fledged National Bureau to have an integrated approach for the collection, conservation, and eventual utilization of genetic resources of finfishes, crustaceans such as shrimps, prawns, lobsters and other shell fishes of India. The Project was initiated with Late Dr. Arun J. Jhingran as Project Director and this was followed by the appointment of Dr. P. Das as Director heading the Bureau at Allahabad (later shifted to its permanent campus at Lucknow) and with a research unit at CMFRI campus, Cochin. I was happy when one of my erstwhile colleagues, Dr. A. G. Ponniah succeeded Dr. P. Das as Director. Thanks to ICAR, I have also been associating with the Bureau as Chairman of the Research Advisory Committee during the last few years. The successive directors including Dr. W. S. Lakra and for an interim period Dr. D. Kapoor and the staff have all contributed towards the growth and enhancing the vision of this great Institution.

Nevertheless, I must mention as an anecdote, the international reaction to the setting up of a Bureau of Fish Genetic Resources in India. At the ACMRR Meetings with fishery experts at FAO, Rome in 1981 when I mentioned about ICAR's plan about setting up a Bureau of Fish Genetic Resources as we already had a Bureau of Plant Genetic Resources, the reaction was one of derision: "*A bureau of fish genetics*!! *In India??" "What was the need for India to have a Bureau of Fish Genetics, unheard of in other countries?"* Looking back, we should all appreciate the creative thinking and vision of ICAR which has made the Bureau a reality. So the phylogeography of NBFGR - originated at Cochin, migrating northwards to Allahabad and thence to the North West to Lucknow! Today we see the sea change brought about by NBFGR and its achievement in the areas of its mandate.

I wish the institution all success in implementing its Vision 2020 as well as take up creative and innovative research and devote attention on core areas of its mandate. May the Bureau grow from strength to excellence and see many more jubilees.

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